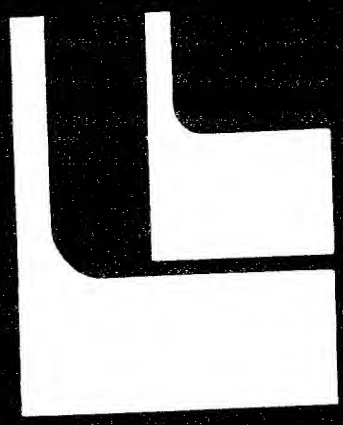
Twenty-second Edition

Harper's Biochemistry



a LANGE medical book

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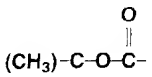
To locate disulfide bonds, peptides from untreated and from reduced or oxidized protein are separated by 2-dimensional chromatography or by electrophoresis and chromatography (fingerprinting). Vizualization with ninhydrin reveals **2 fewer peptides** in the digest from untreated protein and **one new peptide** in the digest from treated protein. With knowledge of the primary structure of these peptides, the positions of disulfide bonds can then be inferred.

PEPTIDES ARE SYNTHESIZED BY AUTOMATED TECHNIQUES

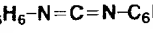
Fig 5–12, which illustrates synthesis of a representative dipeptide A-B by the Merrifield solid-phase technique, summarizes all the reactions required to synthesize a peptide of any desired length. These steps in the procedure are:

1. Block the N-termini of amino acid A (open symbol) and amino acid B (shaded symbol) with the *t*-butyloxycarbonyl [*t*-BOC] group (■):



forming *t*-BOC-A and *t*-BOC-B.

2. Activate the carboxyl group of *t*-BOC-B with dicyclohexyl carbodiimide (DCC) (▶):



3. React the carboxyl group of amino acid A (which will become the C-terminal residue of the peptide) with an activated, insoluble polystyrene resin ( ).

4. Remove the blocking group from *t*-BOC-A with room temperature trifluoroacetic acid (TFA, F_3C-COOH).

[**Note:** In practice, steps 3 and 4 may be omitted since resins with any given *t*-BOC-amino acid connected via an ester bond to a phenylacetamidomethyl (PAM) "linker" molecule attached to the polystyrene resin are commercially available.]

5. Condense the activated carboxyl group of *t*-BOC-B with the free amino group of immobilized A.

6. Remove the *t*-BOC blocking group with TFA (see step 4).

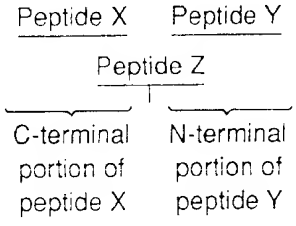


Figure 5–11. The overlapping peptide Z is used to deduce that peptides X and Y are present in the original protein in the order X → Y, not Y → X.

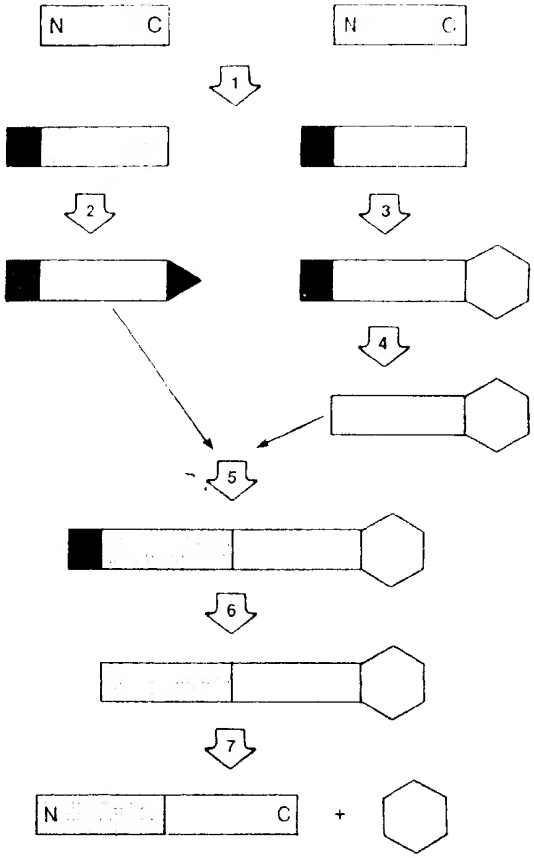


Figure 5–12. Symbolic representation of the synthesis of a generic dipeptide by the solid-phase synthesis technique pioneered by Merrifield. See accompanying text for explanations of symbols.

7. Liberate the dipeptide A-B from the resin particle by treating at −2 °C with HF in dichloromethane.

The initial achievements of the Merrifield technique were the synthesis of the A chain (21 residues) and B chain (30 residues) of insulin in 11 days and of the enzyme pancreatic ribonuclease in 18% overall yield. Subsequent improvements have reduced the time for synthesis of a peptide bond to about 1 hour and have increased yields significantly. This has initiated new prospects, not only for confirming *de novo* synthesis of the primary structures of proteins, but for immunology, for producing vaccines and polypeptide hormones, and conceivably also for treating selected inborn errors of metabolism.

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